Axons of sacral preganglionic neurons in the cat: I. Origin, initial segment, and myelination

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Abstract
Parasympathetic preganglionic neurons in the cat sacral spinal cord innervate intraspinal neurons and pelvic target organs. Retrograde tracing studies have revealed little of the morphology of their axons including their origin, initial segments, or their myelin, due to methodological limitations. Intracellular labeling of single neurons with neurobiotin or HRP has overcome these problems. Axons were studied in 24 preganglionic neurons. In 21 neurons the axon originated as a branch of a dendrite, without a detectable axon hillock, at distances from the soma ranging from 10 to 110 \( \mu m \) (average 34.1 \( \mu m \)). In 3 neurons the axon was derived from the soma. Initial segments, present in all cells, ranged from 15 to 40 \( \mu m \) (average 26.8 \( \mu m \)). Nearly all axons followed the initial segment with unmyelinated segments that varied between 59 to 630 \( \mu m \), followed by myelin and nodes of Ranvier. Internodal distances were variable and relatively short (average 93 \( \mu m \)). Axonal diameters measured over the intraspinal course in 18 axons averaged 1.3 \( \mu m \) (range 0.6–2.4 \( \mu m \)) and were relatively constant compared with other neurons. Spine-like protrusions were observed on the initial segments of 12 cells. Axon collaterals originated from unmyelinated sections and nodes of Ranvier. Antidromic action potentials showing initial segment, soma-dendritic inflections, did not differentiate between soma-derived and dendrite-derived axons. The data suggest that axons originating from a dendrite are the normal structure of preganglionic neurons in the lateral sacral parasympathetic nucleus. It is proposed that the particular structure of these axons may be part of a timing mechanism that coordinates preganglionic neurons with other spinal neurons involved in target organ reflexes.

Introduction
Preganglionic neurons (PGN) located in the sacral parasympathetic nucleus in the cat innervate the bladder, urethra, colon, and sex organs (de Groat et al., 1978, 1979; Morgan et al., 1979; Nadelhaft et al., 1980). In the lateral band of this nucleus, in lateral lamina VII, the majority (66%) of PGN are thought to innervate the urinary bladder (de Groat & Ryall, 1969; Morgan et al., 1979). Many retrograde tracing studies have been conducted on the PGN in this region, but relatively little axon morphology could be established. It was clearly demonstrated that axons were labeled as they could be seen entering the ventral roots and were followed into the ventral horn. But tracing the axons to specific sites on the cells of origin was a problem because they became indistinguishable from the ventral dendrites of these cells. Thus, basic data regarding the point of origin of the axon from the cell, the presence of an axon hillock, and the morphology of the initial segment of these neurons, and details of myelination were unknown. To resolve these gaps in our knowledge, as well as other important features including the presence of axon collaterals (AC) and the dendritic morphology of these neurons, a series of single cell injection studies of parasympathetic PGN was undertaken (Morgan et al., 1991, 1993; Morgan & Ohara, 2001). The present report describes the origins and intraspinal morphology of the axons from these PGN.

Methods
The methods used in these experiments are presented here very briefly but have been described in detail elsewhere (Morgan et al., 1993; Morgan & Ohara, 2001). In general, the neurons in this study were obtained by intracellular injection of neurobiotin or HRP in deeply anesthetized cats. The methods of anesthesia, surgery, and euthanasia were approved by the Animal Care and Use Committee at Eastern Virginia Medical School.

Surgery, Recording, Cell Injection
For the electrophysiological recording and cell injection the sacral ventral roots were transected near the dorsal root ganglia, placed on platinum-iridium wire electrodes, and stimulated antidromically with square wave pulses.
0.025 millisecond pulses, 4 cycles/second at 1–15 V. Intracellular recordings were made with fiber-filled glass electrodes beveled to 60–130 MΩ and filled with 2% neurobiotin (Vector) in 0.5 M KCl, pH 7.4; or 4% HRP (Sigma type VI) in 0.05 M Tris buffer and 0.5 M KCl, pH 7.9. Preganglionic neurons were identified by antidromic activation of their ventral roots and conduction velocities between 4 and 16 M/sec. Iontophoretic injections of tracer were made using positive square wave pulses, 200 milliseconds duration, 3–10 nA and applied over 3–15 minutes. Electrophysiological data were recorded on VHS tape with a four channel recorder (Vetter Model 420) and Polaroid images of the oscilloscope screen.

TISSUE PROCESSING

Serial vibratome sections of spinal cord were cut at a nominal thickness of 50 µm and processed with an avidin-biotin HRP reaction, counterstained with toluidine blue, and mounted on glass slides for visualization at the light microscopic level.

Selected alternate sections were processed by staining in 1.5% reduced osmium followed by 1% uranyl acetate and embedding in EPON for electron microscopy. Tissue for use in this study was further cut in ultrathin sections, mounted on Butvar-coated slot grids and examined with a JEOL 1200 TEM.

MICROSCOPY

All dendrites, somata, central axons, and axon collaterals were completely traced using a Leitz microscope equipped with NeuroLucida software (Microbrightfield), a motorized stage, and Panasonic video camera. The dendrites and axon collaterals have been described in detail elsewhere (Morgan & Ohara, 2001; Morgan, 2002). All dendrites, somata, and axons were also traced by hand with a camera lucida attachment to the microscope and entered onto pencil and paper drawings. Measurements of axon diameters and nodes of Ranvier were made with 100 × objective lens on a Nikon Microphot microscope with a Panasonic video camera projecting to a 19" computer monitor. The images were calibrated with a micrometer scale on the microscope, projected to the monitor and measured with a plastic ruler directly from the monitor. In this system 1 mm on the screen represented 0.2 µm. The smallest measurement was 0.1 µm. There was no correction made for shrinkage.

Color images of the axons were captured with a frame grabber and stored as *.tif files. Images used in the figures were altered by converting them to gray scale and improving their contrast. Some of the figures were also altered by combining in Adobe Photoshop parts of serial images taken at different focal planes. Arrows and text were added in Micrografx Designer and prints were made with a Hewlett-Packard 882C printer.

CRITERIA FOR IDENTIFICATION OF A DENDRITE

The determination of the border between the soma and dendrite in these injected neurons was an estimate based upon their similarity to Nissl stained, presumptive PGN in the same region and in the same and adjacent sections. In the Nissl stained cells, in the autonomic nucleus, as elsewhere, the soma border is marked by a significant reduction in the Nissl stain and a loss from view of the dendrite. In the neurobiotin and HRP stained cells the soma borders were placed in similar locations and the base diameters of all the dendrites of each cell were measured. Measurements of the distance from the soma to the origin of the axon were made from this border and are referred to as the length to origin.

Results

NUMBERS OF LABELED PGN, AXON VERIFICATION AND INTRASPINAL COURSE

Preganglionic neurons in the sacral parasympathetic nucleus in S2 and S3 were identified electrophysiologically by antidromic stimulation and conduction velocities in the range of 3.8 to 15.9 M/sec. Axons were identified in 24 PGN injected with neurobiotin or HRP, in nine male and seven female cats. No sex differences were observed among these cells and they have thereafter all been treated equally.

Axons were positively identified and differentiated from dendrites by meeting one or more of the following criteria. In ten cells (Table 1) the axons were traced continuously from the labeled cell through the ventral horn to the pia or into a ventral rootlet; in ten other cells the axons were traced into the ventral funiculus; in four cells the axon was traced deep into the ventral horn and in one cell the axon was traced into lamina VII. For all of these cells, but especially the latter two categories, confirmation as axons was supported by the labeled neurite maintaining a constant, small diameter and by the presence of nodes of Ranvier and axon collaterals.

Eighteen of these axons were entered into computer-assisted drawings. Figure 1 is a composite drawing of ten of these axons superimposed on the outline of a single section of the gray matter to show their intraspinal course. The somata, shown without dendrites, are clustered in the sacral parasympathetic nucleus in lateral lamina VII. The axons all leave ventrally and follow the lateral edge of the gray matter in a path about 150 µm wide. Upon reaching the lower ventral horn a few axons take a brief medial course before coming back to join the others entering the ventral funiculus and then the ventral roots. Figure 1B is a parasagittal rotation showing the axons leaving the cell bodies in the rostral direction for 50 to 150 µm before reversing direction. Near the level of the ventral horn–ventral funiculus border many of the axons are at the same longitudinal plane as their soma and they continue caudally in the ventral funiculus to exit the cord 50 to 150 µm caudal to the soma. This same pattern is also reflected in the horizontal rotation in Figure 1C. The axons of the other cells not included in this drawing followed a similar course but were excluded from the figure for clarity. The average length of the axons within the gray matter was 1092 ± 34 µm. For the 10 axons traced through the ventral funiculus to the pia and ventral roots, the average length was 1691 ± 64 µm.